Applied Chemistry of Western and Chinese Medicines

1

Chemistry and Mechanisms of different kinds of Drugs

Antibiotics

- Antibiotics (抗生素) (抗細菌藥), also called antibacterial agents, are a type of antimicrobial drug used in the treatment and prevention of bacterial infections.
- The success of antibacterial agents
  - They can act selectively against bacterial cells rather than animal cells.

2

Year	Types of antibiotics in usage
1910	Salvarsan (arsenic-containing compound)
1934	Proflavine
1940	Penicillin
1945	Bacitracin (peptide antibiotics)
1948	Chlortetracycline (tetracycline antibiotics)
1952	Erythromycin (macrolide antibiotics)
1955	Cephalosporin C (β-lactam antibiotics)

3

- Many antibacterial agents are now available.
  - Majority of bacterial diseases have been under control.
  - e.g. syphilis (梅毒), tuberculosis (肺癆), typhoid (傷寒).
- This represents a great achievement for medicinal chemistry.
- However bacteria, such as *Staphylococcus aureus* (金黃葡萄球菌), have the ability to gain resistance to known drugs.
  - So the searching for new drugs is never-end.

4

The bacterial cell

- Bacterial cells and animal cells differ both in their structure and in their biosynthetic pathways.

	Bacteria l cell	Animal cell
Nucleus	No defined nucleus	Defined nucleus
Organelles (mitochondria, endoplasmic reticulum)	Simple	Complex
Cell wall	Yes	No
Cell membrane	Yes	Yes
Biochemistry	have to synthesize essential vitamins	can acquire intact from food

5

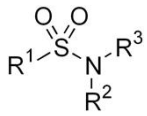
Mechanisms of antibacterial action

- There are five main mechanisms for antibacterial action.
  - Inhibition of cell metabolism
  - Inhibition of bacterial cell wall synthesis
  - Interactions with the plasma membrane
  - Disruption of protein synthesis
  - Inhibition of nucleic acid transcription and replication

6

Inhibition of cell metabolism

- Antibacterial agents which inhibit cell metabolism are called antimetabolites.
- They inhibit the metabolism of a microorganism, but not the metabolism of the host.
- They can do this by inhibiting an enzyme-catalyzed reaction which is present in the bacterial cell but not in animal cell.
- The best known example is sulphonamides (磺胺).



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Inhibition of bacterial cell wall synthesis

- The cell wall is crucial to the bacterial cell's survival.
  - Bacteria have to survive a wide range of environments and osmotic pressures.
- If a bacterial cell lacking a cell wall was placed in an aqueous environment with a low concentration of salts,
  - water would freely enter the cell as a result of osmotic pressure.
  - The cell would swell and eventually burst.
  - This is called lysis.

8

- This type of antibacterial agents lead to bacteria cell lysis and death.
- The best known example is penicillins (盤尼西林) (青黴素).

Interactions with the plasma membrane

- Some antibacterial agents interact with the plasma membrane of bacterial cells
  - ➢ to affect membrane permeability.
  - ➢ This has fatal results for the cell.
- Examples are polymyxins (多粘菌素).

9

Disruption of protein synthesis

- Essential proteins and enzymes required for the survival of bacterial cell cannot be made.
- Examples are tetracyclines (四環素類抗生素), rifamycins (利福黴素), aminoglycosides (氨基糖苷類抗生素) and chloramphenicol (氯黴素).

Inhibition of nucleic acid transcription and replication

- Antibacterial agents prevent cell division and/or the synthesis of essential protein.
- Examples are nalidixic acid and proflavine (原黃素).

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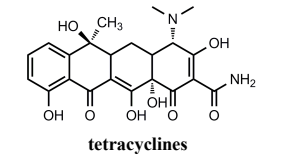
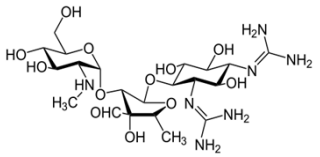
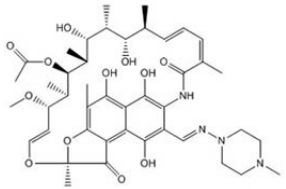


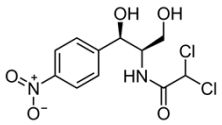
tetracyclines



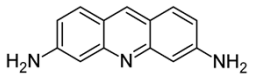
aminoglycosides



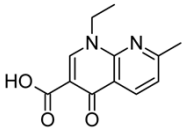
rifamycins



chloramphenicol



proflavine



nalidixic acid

11

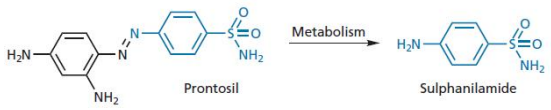
Antibacterial agents act against cell metabolism (antimetabolites)

Sulphonamide

- The best example of antibacterial agents acting as antimetabolites are the sulphonamides.
- The sulphonamide story began in 1935
  - ➢ when it was discovered that a red dye called prontosil.
  - ➢ However no antibacterial effect was observed in lab.

12

- It was discovered that
  - prontosil was metabolized by bacteria present in the small intestine of the test animal.
  - to give a product called sulphanilamide.



- Thus prontosil was an early example of a prodrug.

13

- Sulphanilamide was synthesized in the laboratory and
  - became the first synthetic antibacterial agent found to be active against a wide range of infections.

Structure–activity relationships

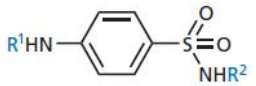
- The synthesis of a large number of sulphonamide analogues led to the following conclusions.
  - The *para*-amino group is essential for activity and must be unsubstituted (i.e. R^1 = H).
    - ✓ The only exception is when R^1 = acyl (i.e. amides).

14

    - ✓ The amides themselves are inactive but can be metabolized in the body to regenerate the active compound.
    - ✓ Hence, amides can be used as sulphonamide prodrugs.
  - The aromatic ring and the sulphonamide functional group are both required.
  - Both the sulphonamide and amino group must be directly attached to the aromatic ring.
  - The aromatic ring must be *para*-substituted only.
    - ✓ Extra substitution eliminates activity for steric reasons.

15

  - The sulphonamide nitrogen must be primary or secondary.
  - R^2 is the only possible site that can be varied in sulphonamides.



16

Applications of sulphonamides

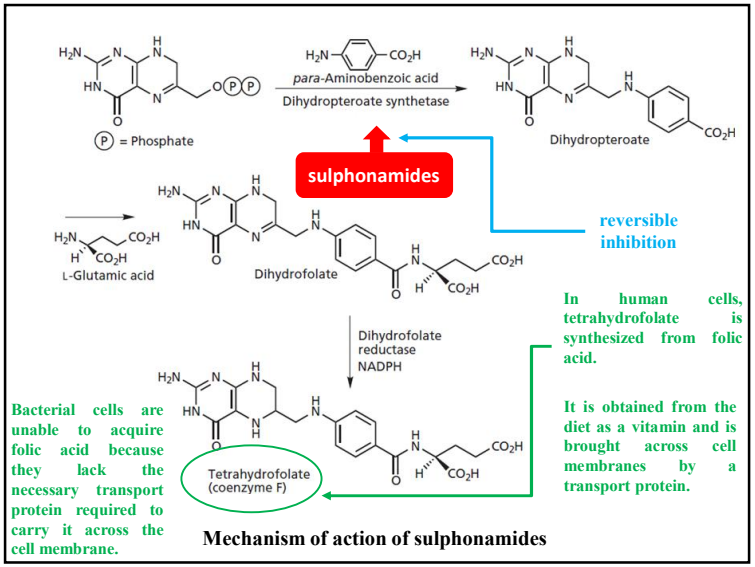
- Before the appearance of penicillin
  - the sulpha drugs were the drugs of choice in the treatment of infectious diseases.
- The sulpha drugs presently have the following applications in medicine:
  - Treatment of urinary tract infections.
  - Eye lotions.
  - Treatment of infections of mucous membranes.
  - Treatment of gut infections.

17

Mechanism of action of sulphonamides

- The sulphonamides act as
  - competitive enzyme inhibitors of dihydropteroate synthetase and
  - block the biosynthesis of tetrahydrofolate in bacterial cells.
- Tetrahydrofolate is important in both human and bacterial cells,
  - because it is an enzyme cofactor that provides one carbon units for the synthesis of the pyrimidine nucleic acid bases required for DNA synthesis.

18



Mechanism of action of sulphonamides

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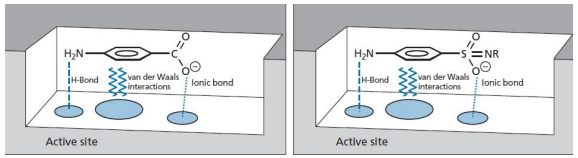
- Sulphonamides act as inhibitors by mimicking *p*-aminobenzoic acid (PABA)
  - one of the normal substrate for dihydropteroate synthetase.



- The sulphonamide molecule is similar in structure to PABA that
  - The enzyme is fooled into accepting it into its active site.

20

- Once it is bound,
  - the sulphonamide prevents PABA from binding.
- Hence dihydropteroate is no longer synthesized.
- Sulphonamides are competitive enzyme inhibitors so inhibition is reversible.
- If pyrimidine and DNA synthesis is blocked,
  - then the cell cannot grow and divide.
- Sulphonamides do not actively kill bacterial cells.

21

- However they prevent the cells growing and multiplying.
  - This gives the body's own defence systems enough time to gather their resources
  - and wipe out the invader.
- Antibacterial agents which inhibit cell growth are classed as bacteriostatic.
- Because sulphonamides rely on a healthy immune system to complete the job they have started
  - they are not recommended for patients with a weakened immune system.

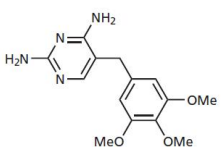
22

- This includes
  - people with AIDS,
  - patients who are undergoing cancer chemotherapy,
  - have had an organ transplant and are taking immuno-suppressant drug.
- The success of sulphonamides is due to two metabolic differences between mammalian and bacterial cells.
  - Bacteria have a susceptible enzyme which is not present in mammalian cells.
  - Bacteria lack the transport protein that would allow them to acquire folic acid from outside the cell.

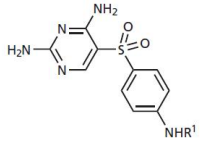
23

Examples of other antimetabolites

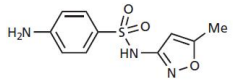
- Other antimetabolites in medical use include
  - trimethoprim and
  - a group of compounds known as sulphones.



Trimethoprim (antimalarial)



Sulphones (anti-leprosy)



Sulphamethoxazole

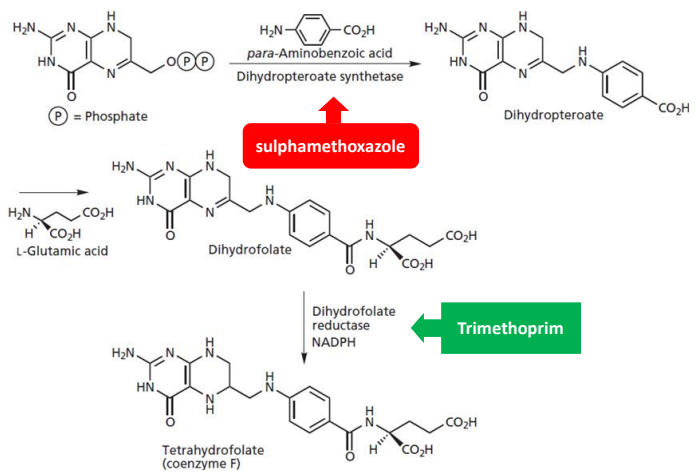
24

- Trimethoprim is an orally active diaminopyrimidine structure
  - which has proved to be a highly selective antibacterial and antimalarial agent.
- It acts against dihydrofolate reductase
  - the enzyme which carries out the conversion of dihydrofolate to tetrahydrofolate
  - leading to the inhibition of DNA synthesis and cell growth.
- Trimethoprim is often given in conjunction with the sulphonamide sulphamethoxazole.

25

  - A preparation called cotrimoxazole.
- The sulphonamide inhibits the incorporation of PABA into dihydropteroate.
  - while trimethoprim inhibits dihydrofolate reductase.
- Therefore, two enzymes in the one biosynthetic route are inhibited.
- This is a very effective method of inhibiting a biosynthetic route and
  - has the advantage that the doses of both drugs can be kept down to a safe level.

26



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- The sulphones are the most important drugs
  - used in the treatment of leprosy.
- It is believed that they inhibit the same bacterial enzyme inhibited by the sulphonamides
  - i.e. dihydropteroate synthetase

28

Antibacterial agents which inhibit cell wall synthesis

Penicillins

- In 1928, Fleming noted that a bacterial culture that had been left several weeks open to the air
  - had become infected by a fungal colony.
- Of more interest was the fact that
  - there was an area surrounding the fungal colony where the bacterial colonies were dying.

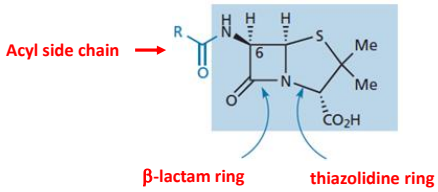
29

- He concluded that the fungal colony was producing an antibacterial agent
  - which was spreading into the surrounding area.
- However he could not isolate the product.
- The problem of isolating penicillin was eventually solved in 1938 by Florey and Chain by using processes
  - such as freeze-drying and
  - chromatography.

30

Structures of benzylpenicillin and phenoxymethylpenicillin

- Penicillin contains a highly unstable looking bicyclic system
  - consisting of a four-membered β-lactam ring fused to a five-membered thiazolidine ring.



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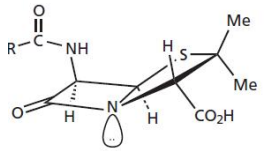
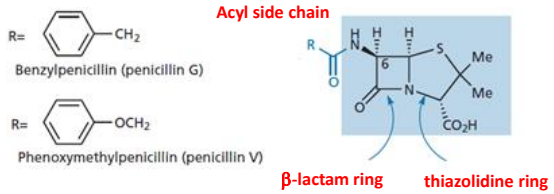


- The acyl side chain (R) varies, depending on the components of the fermentation medium.
- Fermentation medium contains high levels of phenylacetic acid ($PhCH_2CO_2H$)
  - gives benzylpenicillin (penicillin G; R = benzyl).

32

- Fermentation medium containing phenoxyacetic acid ($PhOCH_2CO_2H$)
  - gives phenoxymethylpenicillin (penicillin V; R = $PhOCH_2$).

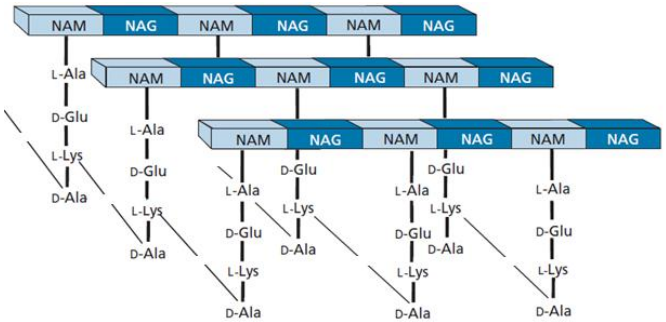


33

Mechanism of action of penicillin

- The cell wall is a peptidoglycan structure.
  - It is made up of peptide and sugar units.
- The structure of the wall consists of a parallel series of sugar backbones containing two types of sugar.
  - *N*-acetylmuramic acid (NAM)
  - *N*-acetylglucosamine (NAG)
- Peptide chains are bound to the NAM sugars and
  - it is interesting to note the presence of D-amino acids in these chain.

34



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- In the final stage of the cell wall biosynetheis,
  - the peptide chains are linked together by the displacement of D-alanine from one chain by glycine in another.
- The final cross-linking reaction is inhibited by penicillin.
  - This leads to a cell wall framework that is no longer interlinked.
  - The wall becomes fragile and can no longer prevent the cell from swelling and bursting.
- The enzyme responsible for the cross-linking reaction is known as the transpeptidase enzyme.

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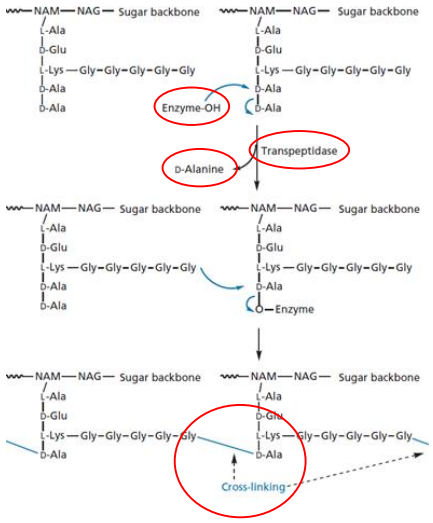
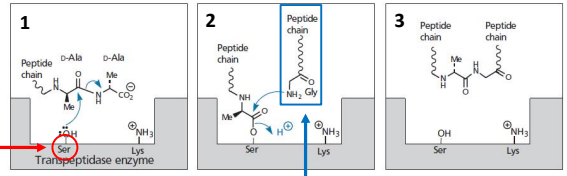


37

1. Serine acts as a nucleophile to split the peptide bond between the two unusual D-alanine units on a peptide chain.

The terminal alanine departs the active site, leaving the peptide chain bound to the active site.

3. The pentaglycyl displaces the alanine group from serine and linking the two chains together.



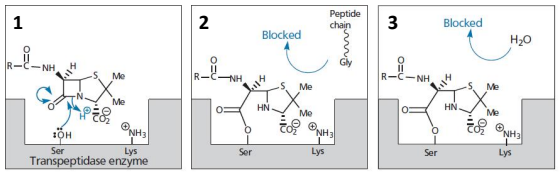
2. The pentaglycyl moiety of another peptide chain now enters the active site and the terminal glycine forms a peptide bond to the alanine group

38

1. Penicillin has a conformation which is similar to the transition-state conformation taken up by the D-Ala-D-Ala moiety during the cross-linking reaction

The enzyme mistakes penicillin for D-Ala-D-Ala and binds it to the active site.

Once bound, penicillin is subjected to nucleophilic attack by serine



The enzyme can attack the β-lactam ring of penicillin and cleave it in the same way as it did with the peptide bond.

2. However, penicillin is cyclic so the molecule is not split in two and nothing leaves the active site.

3.Subsequent hydrolysis of the ester group linking the penicillin to the active site does not take place either, as the penicillin structure blocks access to the pentaglycine chain or water.

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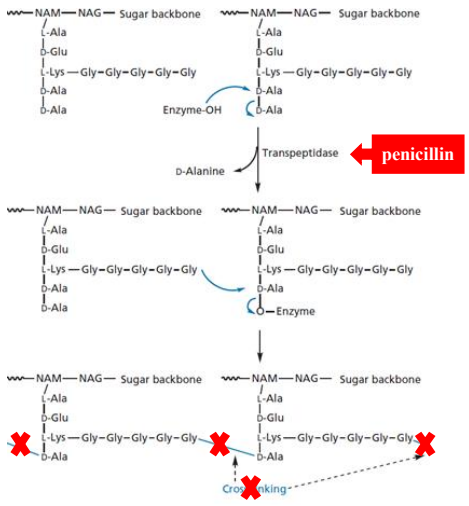

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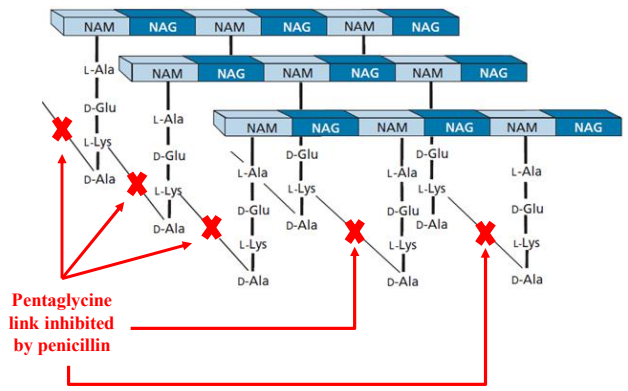


41

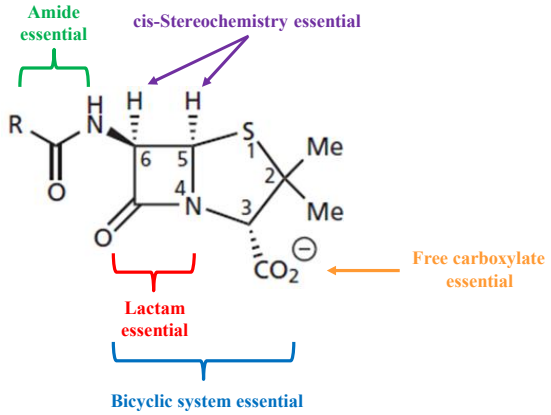
Structure–activity relationships of penicillin

- A large number of penicillin analogues have been synthesized and studied.
- The results of these studies led to the following results.
  - ➢ The strained β-lactam ring is essential.
  - ➢ The free carboxylic acid is essential.
    - ✓ This is usually ionized and penicillins are administered as sodium or potassium salts.
    - ✓ The carboxylate ion binds to the charged nitrogen of a lysine residue in the binding site.

42

- The bicyclic system is important.
  - ➢ This confers further strain on the β-lactam ring.
    - ✓ The greater the strain, the greater the activity.
    - ✓ But the greater the instability of the molecule to other factors.
- The acylamino side chain is essential.
- Sulphur is usual but not essential
- The stereochemistry of the bicyclic ring with respect to the acylamino side chain is important

43



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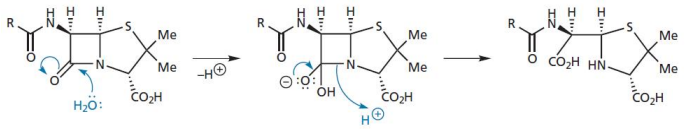
Penicillin analogues

- The penicillin analogues proved successful in tackling the problems of
  - ➢ acid sensitivity,
  - ➢ β-lactamase sensitivity and
  - ➢ limited breadth of activity.



Ring-opening of the β-lactam ring under acidic conditions

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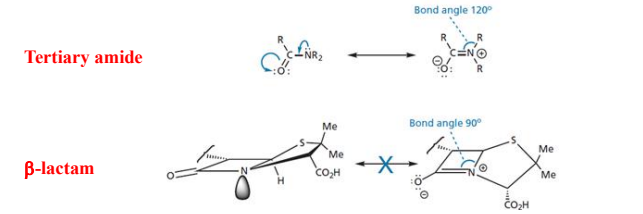

Comparison of *tertiary amide and β-lactam carbonyl groups.*

- Acid resistant penicillin
  - ➢ Phenoxymethylpenicillin (penicillin V)
    - ✓ has an electronegative oxygen on the acyl side chain.

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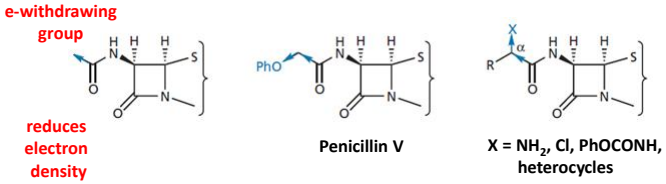
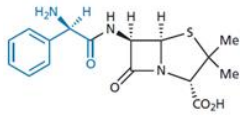


- ➢ Ampicillin
  - ✓ Also has electron-withdrawing substituent on the α-carbon of the side chain.

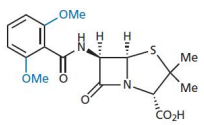


Ampicillin (penbritin)

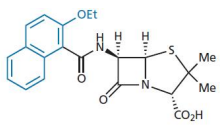
47

- β-Lactamase-resistant penicillins
  - ➢ Methicillin
    - ✓ It was the first effective semi-synthetic penicillin with resistance to β-lactamase enzyme.
  - ➢ Nafcillin
    - ✓ It contains a naphthalene ring which acts as its steric shield.
  - ➢ Temocillin
    - ✓ It has a 6-methoxy group present.

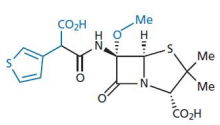
48



Methicllin



Nafcillin



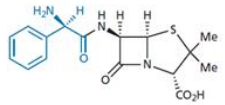
Temocillin

- **Broad-spectrum penicillins**
  - **The spectrum of activity shown by any penicillin depends on**
    - ✓ its structure,
    - ✓ its ability to cross the cell membrane of Gram-negative bacteria,
    - ✓ its susceptibility to β-lactamases,

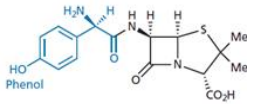
49

- ✓ its affinity for the transpeptidase target enzyme,
- ✓ the rate at which it is pumped back out of cells by Gram-negative organisms.

- **Ampicillin and amoxicillin**



Ampicillin (penbritin)

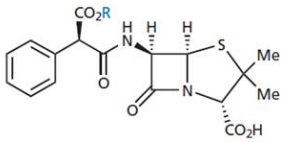


Amoxicillin (Amoxil)

50

- **Carbenicillin**
  - ✓ It was the first example of this class of compounds.
  - ✓ It shows a broad spectrum of activity

    due to the hydrophilic carboxylic acid group on the side chain.

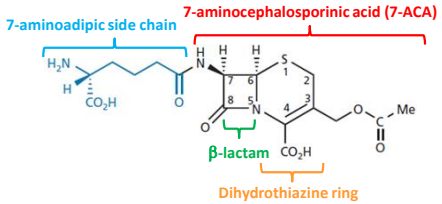


Carbenicillin (R = H)

51

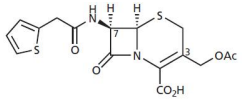
Cephalosporins

- **They are the second major group of β-lactam antibiotics.**
- **The first cephalosporin is cephalosporin C.**
- **The structure has similar to that of penicillin.**
  - **The β-lactam ring is fused to a six-membered dihydrothiazine ring**

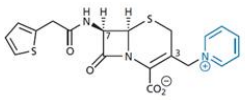


52

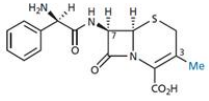
- There are altogether 5 generations of cephalosporins.
  - First-generation cephalosporins
    - ✓ Cephalothin , cephaloridine , cefalexin , and cefazolin.



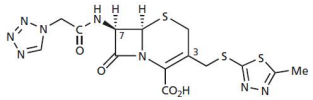
Cephalothin



Cephaloridine



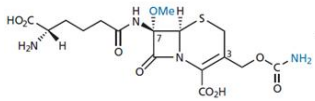
Cefalexin



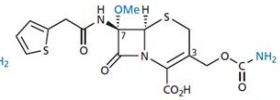
Cefazolin

53

- Second-generation cephalosporins
  - ✓ Cephamycins and cefoxitin



Cephamycins

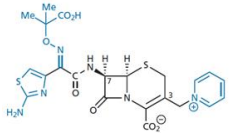


Cefoxitin

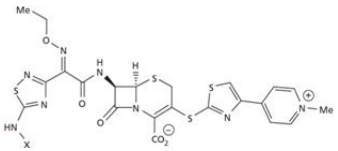
- Third-generation cephalosporins
  - ✓ Ceftazidime , cefotaxime, ceft izoxime , and ceftriaxone.
- Fourth-generation cephalosporins
  - ✓ Cefepime and cefpirome are oximinocephalosporins.

54

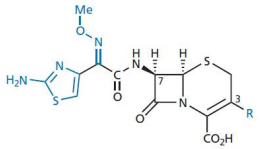
- Fifth-generation cephalosporins
  - ✓ Ceftaroline fosamil

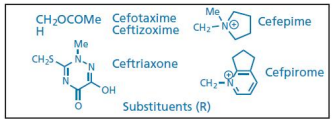


Ceftazidime



Ceftaroline; X = H
Ceftaroline fosamil; X = $P(=O)(OH)_2$



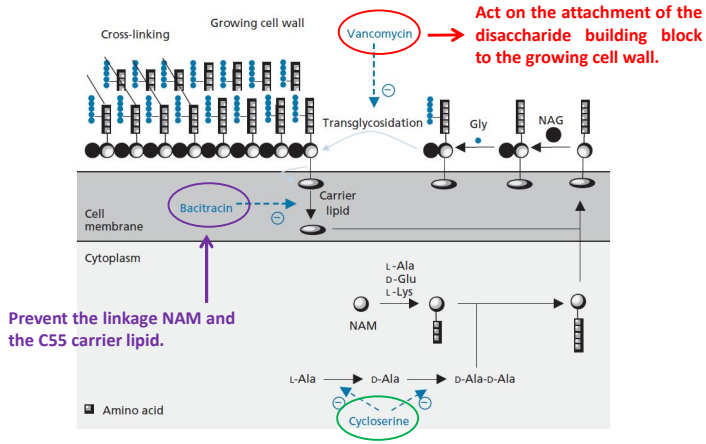


55

Other drugs

- β-Lactams are not the only antibacterial agents that inhibit cell wall biosynthesis.
- The antibacterial agents.
  - Vancomycin,
  - D-cycloserine and
  - bacitracin
  - also inhibit biosynthesis, though at different stages.

56



57

Antibacterial agents act on the plasma membrane structure

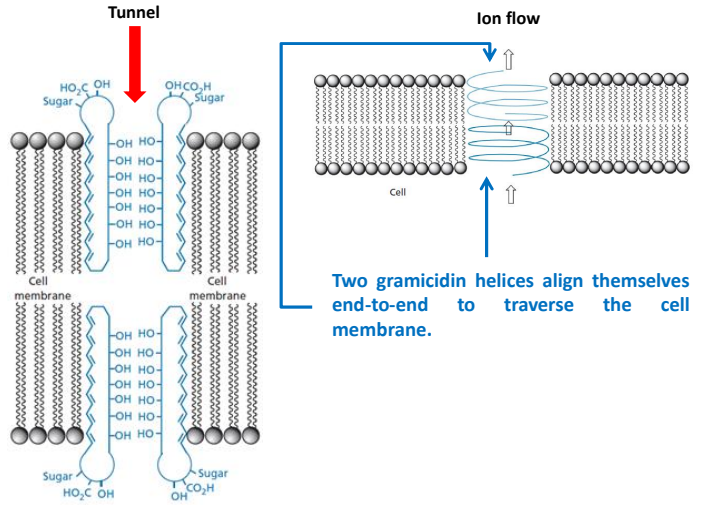
Valinomycin and gramicidin A

- The peptides valinomycin and gramicidin A both act as
  - ion-conducting antibiotics (ionophores) and
  - allow the uncontrolled movement of ions across the cell membrane.

Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NH-CH_2-CH_2-OH

Structure of gramicidin A

58



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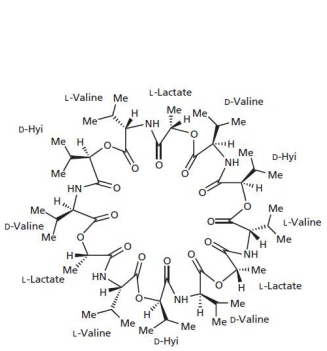
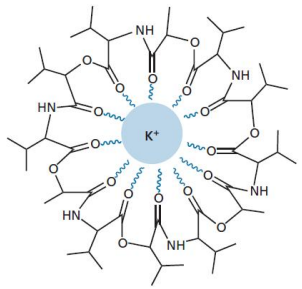


D-Hyi = D-Hydroxyisovaleric acid

Structure of valinomycin



1. Valinomycin can, therefore, collect a potassium ion from the inner surface of the membrane,
2. carry it across the membrane and deposit it outside the cell,
3. thus disrupting the ionic equilibrium of the cell

60

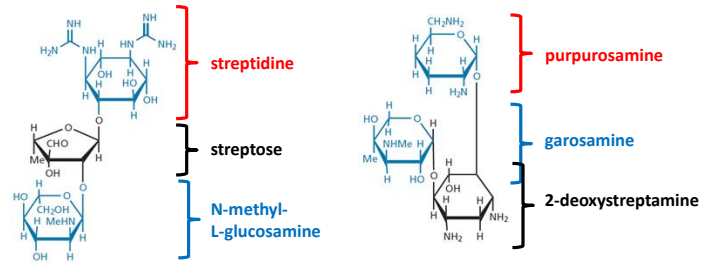
Antibacterial agents which impair protein synthesis: translation

- They all inhibit protein synthesis by binding to ribosomes and inhibiting different stages of the translation process.
- Five antibacterial agents will be discussed.
  - Aminoglycosides (氨基糖苷類抗生素)
  - Tetracyclines (四環黴素)
  - Chloramphenicol (氯黴素)
  - Macrolides (大環內酯)
  - Oxazolidinones (唑烷酮類)

61

Aminoglycosides

- Streptomycin is an example of an aminoglycoside.
  - A carbohydrate structure which includes basic amine groups.



Streptomycin (from *Streptomyces griseus*) Gentamicin C1a

62

- Streptomycin is the next most important antibiotic to be discovered after penicillin.
- Another example gentamicin C1a.
- The drug crosses the cell membrane and
  - bind to bacterial ribosomes to inhibit protein synthesis.
  - The binding is specifically to the 30S ribosomal subunit and prevents the movement of the ribosome along mRNA.

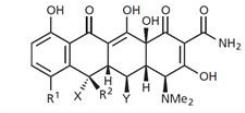
63

Tetracyclines

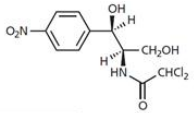
- The tetracyclines are bacteriostatic antibiotics which have a broad spectrum of activity.
- They are the most widely prescribed form of antibiotic after penicillins.
- One of the best known tetracyclines is chlortetracycline (aureomycin).
- Further tetracyclines, such as tetracycline and doxycycline have been synthesized or discovered later.

64

- The tetracyclines inhibit protein synthesis by binding to the 30S subunit of ribosomes and
  - preventing aminoacyl-tRNA from binding.
  - This stops the further addition of amino acids to the growing protein chain.
  - Protein release is also inhibited.



Chlortetracycline (Aureomycin) (R^1 = Cl, R^2 = Me, X = OH, Y = H)
Tetracycline (R^1 = H, R^2 = Me, X = OH, Y = H)
Doxycycline (Vibramycin) (R^1 = H, R^2 = Me, X = H, Y = OH)
Demeclocycline (R^1 = Cl, R^2 = H, X = OH, Y = H)



Tetracyclines Chloramphenicol

65

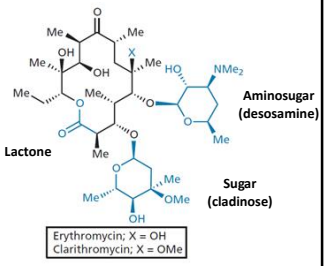
Chloramphenicol

- Chloramphenicol binds to the 50S subunit of ribosomes
  - appears to act by inhibiting the movement of ribosomes along mRNA,
  - probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended.

66

Macrolides

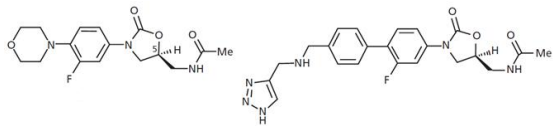
- The best-known example of this class of compounds is erythromycin.
- It is of the safest antibiotics in clinical use.
- Erythromycin acts by binding to the 50S subunit of bacterial ribosomes to inhibit translocation.
- Erythromycin and chloramphenicol bind to the same region of the ribosome.



67

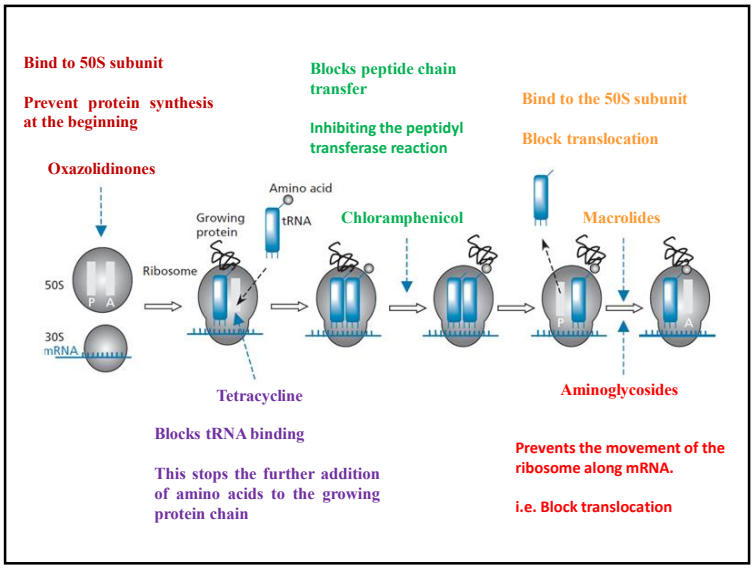
Oxazolidinones

- The oxazolidinones are a new class of synthetic antibacterial agents discovered in recent years.
- They inhibit protein synthesis at a much earlier stage.
- The oxazolidinones bind to the 50S ribosome and prevent this from happening.
  - As a result, translation cannot even start.



Linezolid Radezolid

68

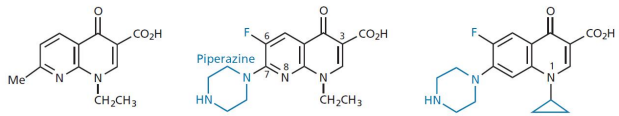


69

Antibacterial agents that act on nucleic acid transcription and replication

➢ Quinolones and fluoroquinolones

- They are particularly useful in the treatment of urinary tract infections and
  - ➢ infections which prove resistant to the more established antibacterial agents.

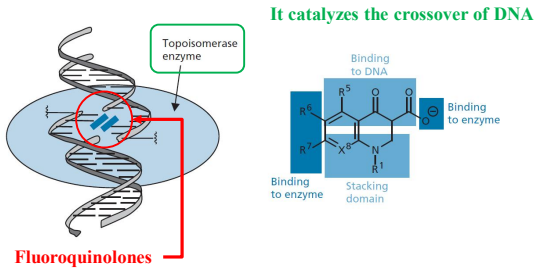


Nalidixic acid | Enoxacin | Ciprofloxacin

Quinolones and fluoroquinolones

70

- They inhibit the replication and transcription of bacterial DNA
  - ➢ by stabilizing the complex formed between DNA and topoisomerases.

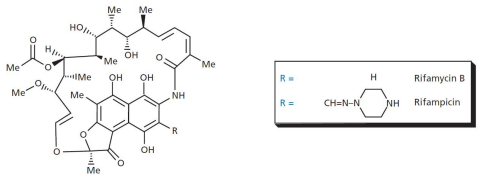


Complex formed between DNA, the topoisomerase enzyme and fluoroquinolones; R^6 = F for fluoroquinolones

71

➢ Rifamycins

- Rifamycin is a semi-synthetic rifamycin made from rifamycin B.
- It inhibits Gram-positive bacteria and works by
  - ➢ binding non-covalently to DNA-dependent RNA polymerase
  - ➢ and inhibiting the start of RNA synthesis.



72

- It is used mainly in the treatment of tuberculosis and staphylococci infections that resist penicillin.
- It is a very useful antibiotic
  - showing a high degree of selectivity against bacterial cells over mammalian cells.
- But it is expensive.

73

Analgesics

- An analgesic (止痛藥) or painkiller is any member of the group of drugs used to achieve analgesia, relief from pain
- Analgesic drugs act in various ways on the peripheral and central nervous systems.
- Analgesics include
  - paracetamol (acetaminophen or simply APAP),
  - salicylates (the nonsteroidal anti-inflammatory drugs, NSAIDs)
  - opioid drugs such as morphine and oxycodone.

74

Opioid analgesics

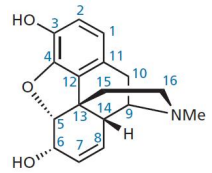
- Opioid analgesics, also known as narcotic analgesics, are pain relievers.
- The term opiates refers to narcotic analgesics that
  - are structurally related to morphine.
- Opioids is the term used to cover all the synthetic, semi-synthetic, naturally occurring, and endogenous compounds that
  - interact with opioid receptors in the body.

75

- The first opioids were extracted from opium
  - Opium is, perhaps, the oldest herbal medicine.

Morphine

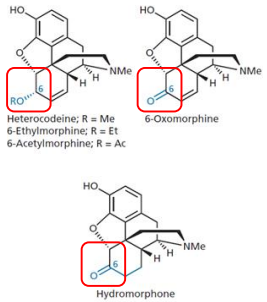
- Opium contains a complex mixture of over 20 alkaloids.
- The principal alkaloid in the mixture and the one responsible for opium's analgesic and sedative activity is
  - morphine.



76

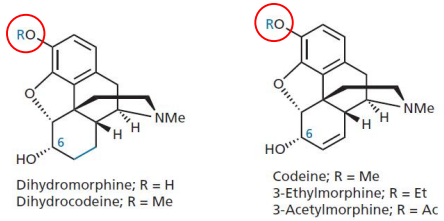
Structure–activity relationships of morphine

- Some conclusion can be made regarding the importance of different functional groups.
- There is no effect on the analgesic activity on the 6-hydroxyl group.
  - heterocodeine
  - 6-ethylmorphine
  - 6-acetylmorphine
  - 6-oxomorphine
  - hydromorphone



77

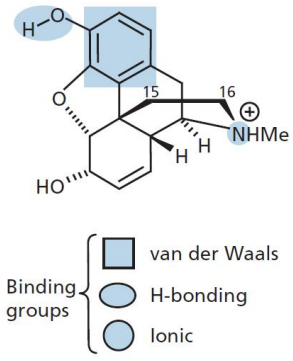
- Analgesic activity drops significantly for codeine, dihydrocodeine and 3-ethylmorphine which
  - indicating the importance of the phenolic group.



78

- The important functional groups for analgesic activity are:
  - the phenol OH group
  - the aromatic ring and
  - the tertiary amine.
- These functional groups play an important role in binding the drug to the binding site by
  - the intermolecular bonding forces.

79

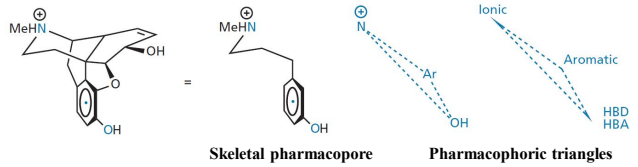


Important functional groups for analgesic activity in morphine

80

- The analgesic activity is not only related to the presence of the important functional groups
  - but to their relative position with respect to each.
  - Pharmacophore
- The pharmacophore summarizes the important binding groups that are
  - required for activity and
  - their relative positions in space with respect to each other.

81



Opioid pharmacophores for morphine and related opioids

The molecular target for morphine: opioid receptors

- It is now known that morphine activates
  - analgesic receptors in the central nervous system (CNS).
  - This leads to a reduction in the transmission of pain signals to the brain.

82

- There are three main types of analgesic or opioid receptor that are activated by morphine.
  - mu (μ) receptor (MOR)
  - kappa (κ) receptor (KOR)
  - delta (δ) receptor (DOR)
- All of them are G-protein-coupled receptors which
  - activate G_i or G_o signal proteins.
- Morphine binds most strongly to the μ receptor.
  - This receptor is responsible for the serious side effects associated with morphine.

83

- Morphine acts as an agonist at all three types of receptor and activate
  - leads to a variety of cellular effects depending on the type of receptor involved.
- These include
  - the opening of potassium ion channels,
  - the closing of calcium ion channels,
  - the inhibition of neurotransmitter release.
- All of which reduce the transmission of pain signals from one nerve cell to another.

84

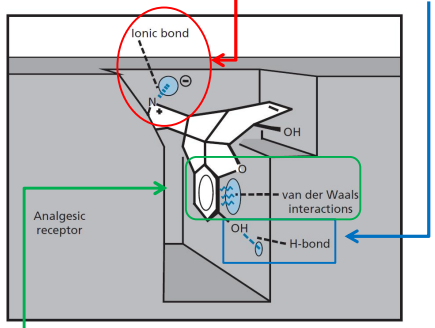
Morphine: pharmacodynamics and pharmacokinetics

- Pharmacodynamics refers to
  - a drug binds to its target and produces a pharmacological effect
- The functional groups are important to the activity of morphine.
  - The amine nitrogen
  - The phenol
  - The aromatic ring

85

The amine nitrogen is protonated and charged allowing it to form an ionic bond with a negatively charged region of the binding site.

The phenol acts as a hydrogen bond donor and forms a hydrogen bond to a hydrogen bond acceptor in the binding site.



The aromatic ring has a defined orientation with respect to the rest of the molecule, allowing van der Waals interactions with the binding site.

Graham, P. L. *An Introduction to Medicinal Chemistry*

86

- Pharmacokinetics refers to
  - the ability of a drug to reach its target
  - and to survive in the body.
- Only a small percentage of the dose actually reaches the analgesic receptors in the CNS
  - blood–brain barrier
- Morphine can cross the blood–brain barrier
  - as the free base then ionize
  - due to the amine group

87

Endogenous opioid peptides and opioids

- Morphine relieves pain by binding to analgesic receptors,
  - which implies that there must be endogenous chemicals which interact with these receptors.
- Enkephalins are example of natural analgesics.
- There are two enkephalins.
  - Met-enkephalin
  - Leu-enkephalin

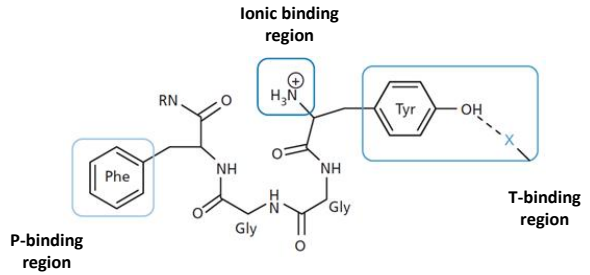
H-Tyr-Gly-Gly-Phe-Met-OH — Met-enkephalin

H-Tyr-Gly-Gly-Phe-Leu-OH — Leu-enkephalin

88

- The tyrosine residue and the aromatic ring of phenylalanine are important for analgesic activity.
  - they act as important binding groups in their interaction with opioid receptors.
- The receptor binding site contains two hydrophobic binding regions.
  - one which interacts with the phenol ring of tyrosine (the T-binding region)
  - one which interacts with the aromatic ring of phenylanine (the P-binding region).

89



90

Antiviral agent

Viruses and viral diseases

- Viruses are non-cellular, infectious agents which
  - take over a host cell in order to survive and multiply.
- Viruses can be transmitted in a variety of ways.
- Those responsible for diseases such as
  - influenza (flu) (流感),
  - chicken pox (水痘),
  - Measles (麻疹),

91

  - Mumps (流行性腮腺炎),
  - viral pneumonia (病毒性肺炎),
  - rubella (德國麻疹)
  - small pox (天花) and
  - severe acute respiratory syndrome (嚴重急性呼吸系統綜合症, 沙士)
  - can be transmitted through the air by an infected host sneezing or coughing.
- Other viruses can be transmitted by means of
  - arthropods or ticks.
  - e.g. yellow fever (黃熱病)

92

- Some viruses are unable to survive for long outside the host and are transmitted through physical contact.
  - AIDS (後天免疫缺乏症, 愛滋病)
  - common cold (感冒)
  - genital herpes (生殖器疱疹)
- Food- or water-borne viruses can lead to
  - hepatitis A and E (甲型肝炎, 戊型肝炎),
  - poliomyelitis (小兒麻痺症)
  - viral gastroenteritis (病毒性腸胃炎)

93

Structure of viruses

- All viruses contain one or more molecules of
  - either DNA or RNA,
  - but not both.
- They can be defined as DNA or RNA viruses.
- Most RNA viruses contain single-stranded RNA (ssRNA),
  - but some viruses contain double-stranded RNA (dsRNA).

94

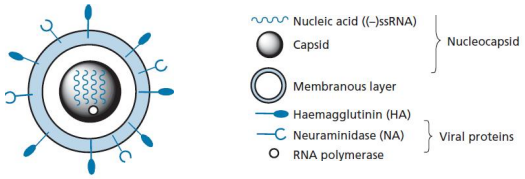
- If the base sequence of the RNA strand is identical to viral mRNA,
  - it is called the positive (+) strand.
- If it is complementary,
  - it is called the negative (-) strand.
- Most DNA viruses contain double-stranded DNA (dsDNA),
  - but a small number contain single-stranded DNA (ssDNA).

95

- The viral nucleic acid is contained and protected within a protein coat called
  - capsid.
- Capsids are made up of protein subunits called protomers which
  - are generated in the host cell
  - and can interact spontaneously to form the capsid in a process called self-assembly.
- Once the capsid contains the viral nucleic acid, the whole assembly is known as
  - nucleocapsid.

96

- **Additional membranous layers of carbohydrates and lipids may surround the nucleocapsid.**
- **The complete structure is known as a virion.**
- **This is the form that the virus takes when it is outside the host cell.**



Diagrammatic representation of the flu virus

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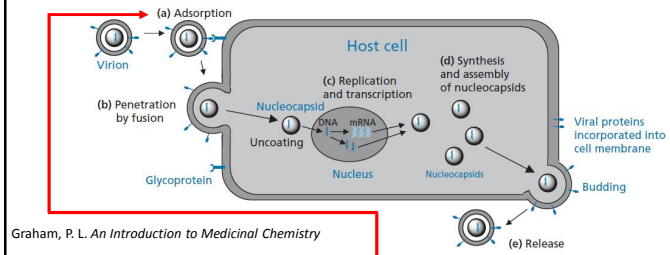
97



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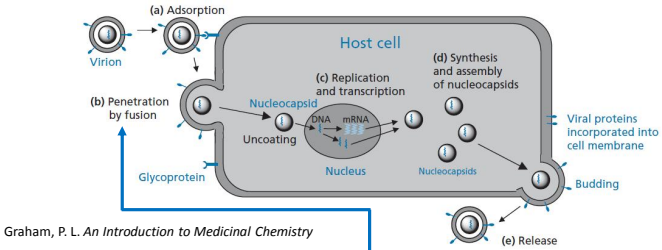
(a) Adsorption

A virion bind to the outer surface of a host cell.

This involves a specific molecule on the outer surface of the virion binding to a specific protein or carbohydrate present in the host cell membrane.

98



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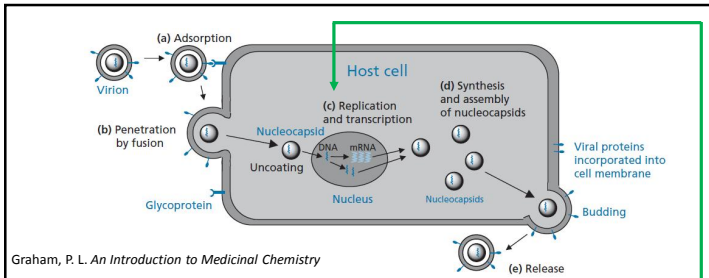
(b) Penetration by fusion

Different viruses introduce heir nucleic acid into the host cell by different methods.

Some inject their nucleic acid through the cell membrane; others enter the cell intact and are then uncoated.

The viral envelope of some virions fuses with the plasma membrane and the nucleocapsid is then introduced into the cell.

99



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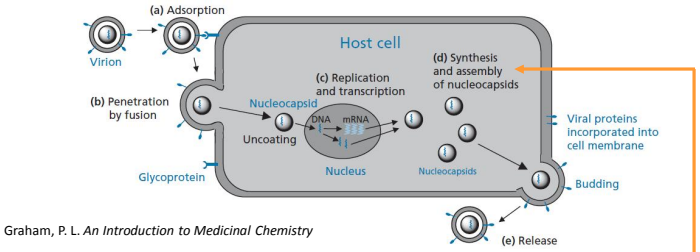
(c) Replication and transcription

Viral genes can be defined as early or late

The mechanism involved varies from virus to virus.

For example, viruses containing negative ssRNA use a viral enzyme called RNA-dependent RNA polymerase to synthesize mRNA which then codes for viral proteins

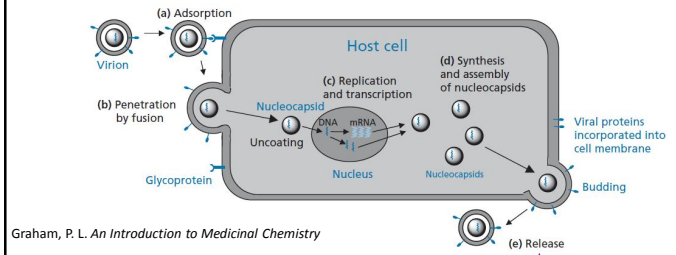
100



(d) Synthesis and assembly of nucleocapsids

Late genes direct the synthesis of capsid proteins and these selfassemble to form the capsid. Viral nucleic acid is then taken into the capsid to form the nucleocapsid.

101



(e) Release

Viruses with envelopes are usually released by a process known as budding

Viral proteins are first incorporated into the host cell's plasma membrane.

The nucleocapsid then binds to the inner surface of the cell membrane and, at the same time, viral proteins collect at the site and host cell proteins are excluded.

The wraps itself round the nucleocapsid and is pinched off plasma membrane containing the viral proteins then from the cell to release the mature virion.

102

General principles of antiviral drugs

- **Good drug targets are proteins which are likely to have the following characteristics:**
  - **They are important to the life cycle of the virus such that**
    - ✓ their inhibition or disruption has a major effect on infection.
  - **They bear little resemblance to human proteins**
    - ✓ hence increasing the chances of good selectivity and minimal side effects.

103

  - **They are common to a variety of different viruses and have a specific region which**
    - ✓ is identical in its amino acid composition.
  - **They are important to the early stages of the viral life cycle which**
    - ✓ means that their disruption/inhibition reduces the chances of symptoms and of the virus spreading through the body.
- **Most antiviral drugs in use today**
  - **disrupt critical stages of the virus life cycle or**
  - **the synthesis of virus-specific nucleic acids.**

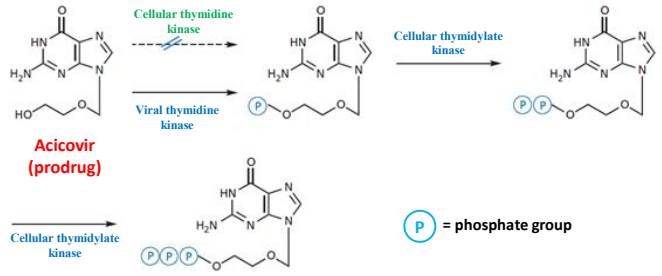
104

Antiviral drugs used against DNA viruses

Inhibitors of viral DNA polymerase

- Aciclovir was discovered and was introduced into the market in 1981.
- However, it lacks the complete sugar ring.
- In virally infected cells, it is phosphorylated in three stages to form a triphosphate which
  - ➢ is the active agent.
  - ➢ So aciclovir is a prodrug.

105



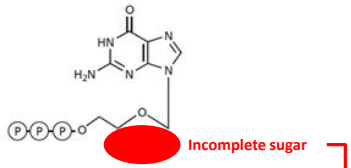
Activation of aciclovir

106

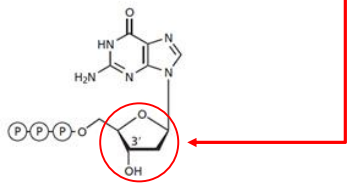
- Aciclovir triphosphate prevents DNA replication in two ways:
  - ➢ It is sufficiently similar to the normal deoxyguanosine triphosphate building block that
    - ✓ it can bind to DNA polymerase and inhibit it.
  - ➢ DNA polymerase can catalyse the attachment of the aciclovir nucleotide to the growing DNA chain.
    - ✓ The drug acts as a chain terminator.
    - ✓ Because the sugar unit is incomplete.

107

- DNA polymerase is an enzyme that catalyze the DNA replication using nucleotide triphosphates as building blocks.



Aciclovir triphosphate



Deoxyguanosine triphosphate

108

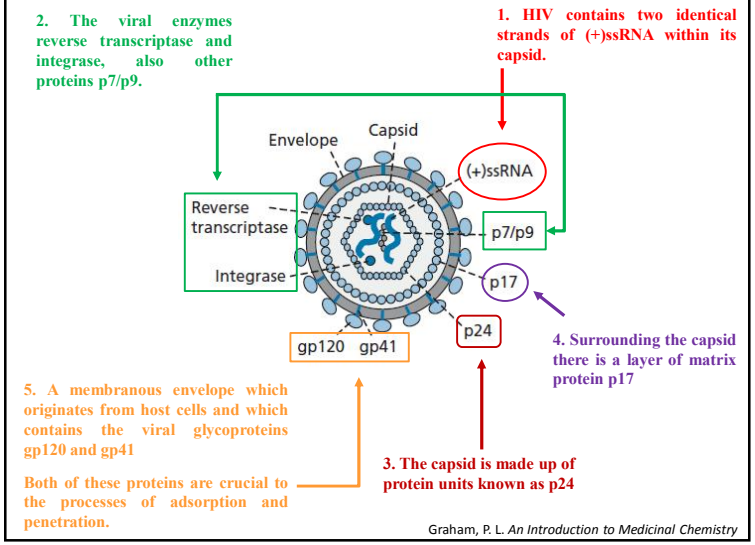
- However the oral bioavailability of aciclovir is quite low (15 – 30%).
  - Various prodrugs were developed to increase water solubility.

Antiviral drugs used against RNA viruses (HIV)

Structure and life cycle of HIV

- In order to understand the antiviral drugs' action,
  - there should be a clear understanding on the structure and life cycle of HIV.

109



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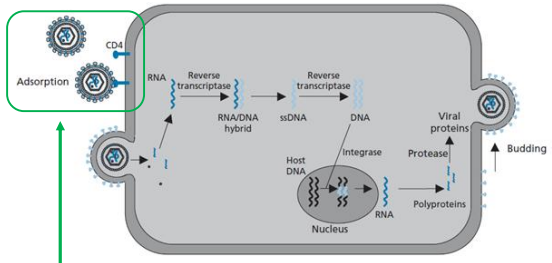
110



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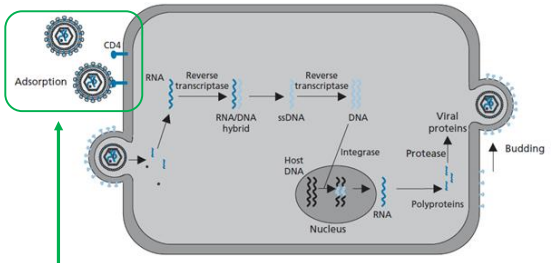
Adsorption

When the virus approaches the host cell, gp120 interacts and binds with a transmembrane protein called CD4 which is present on host T-cell.

The gp120 proteins then undergo a conformational change which allows them to bind simultaneously to chemokine receptors on the host cell.

111



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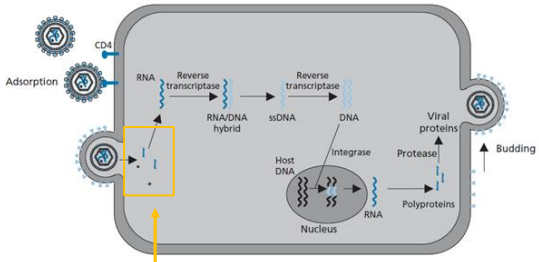
Adsorption

Further conformational changes peel away the gp120 protein allowing the viral protein gp41 to reach the surface of the host cell and anchor the virus to the surface.

The gp41 then undergoes a conformational change and pulls the virus and the cell together so that their membranes can fuse.

112



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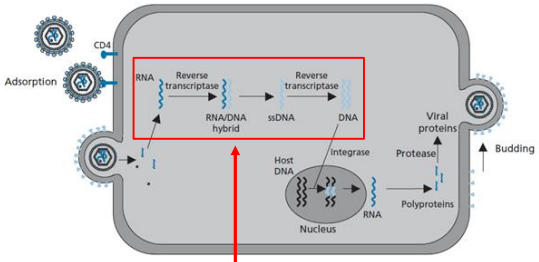
Once fusion has taken place, the HIV nucleocapsid enters the cell.

Disintegration of the protein capsid then takes place, probably aided by the action of a viral enzyme called protease.

Viral RNA and viral enzymes are then released into the cell cytoplasm.

113



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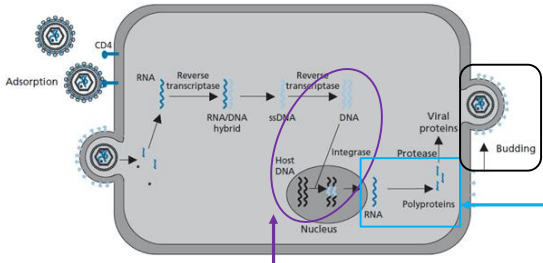
The released viral RNA is not capable of coding directly for viral proteins or of self-replication.

Instead, it is converted into DNA and incorporated into the host cell DNA.

The conversion of RNA into DNA is not a process that occurs in human cells, so there are no host enzymes to catalyse the process.

HIV carries its own enzyme reverse transcriptase to do this.

114



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Proviral DNA is now spliced into the host cell's DNA by the enzyme integrase.

Once the proviral DNA has been incorporated into host DNA, transcription of the viral genes *env, gag* , and *pol* takes place to produce viral RNA.

Some of which will be incorporated into new virions, and the rest of which is used in translation to produce three large, non-functional polyproteins.

Budding then takes place to produce an immature, membrane-bound virus particle.

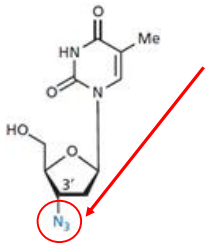
115

Inhibitors of viral reverse transcriptase

- **As the enzyme reverse transcriptase is unique to HIV,**
  - **it serves as an ideal drug target.**
- **The enzyme is still a DNA polymerase and care has to be taken that**
  - **inhibitors do not have a significant inhibitory effect on cellular DNA polymerases.**
- **Various nucleoside-like structures have proved useful as antiviral agents.**
- **However, combination therapy was used.**

116

- Zidovudine was developed originally as an anticancer agent but
  - ➢ was the first drug to be approved for use in the treatment of AIDS.



- The sugar 3′-hydroxyl group has been replaced by an azido group.
- On conversion to the triphosphate, it inhibits reverse transcriptase.
- The triphosphate is attached to the growing DNA chain.

  - ➢ The nucleic acid chain cannot be extended any further do to the present of an azide group in the 3’ position.

117

- Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are generally hydrophobic molecules that
  - ➢ bind to an allosteric binding site.
- The allosteric binding site is separate from the substrate binding site,
  - ➢ So NNRTIs are non-competitive, reversible inhibitors.
- Binding of a NNRTI to the allosteric site,
  - ➢ results in an induced fit which
  - ➢ locks the neighbouring substrate-binding site into an inactive conformation.

118

Inhibitors of other targets

- Unlike the reverse transcriptase inhibitors, the PIs are not
  - ➢ Prodrugs and
  - ➢ do not need to be activated.
- Example: HIV protease enzyme and Saquinavir
- They all inhibit the enzyme protease to make the viral protein.

119

Protease inhibitors

- Antisense agents are being developed to block the production of the HIV protein Tat
  - ➢ which is needed for the transcription of other HIV genes.
- Trecovirsen is a phosphorothioate oligonucleotide
  - ➢ to hybridize with the mRNA derived from the HIV gene *gag* to prevent its translation into HIV proteins.
- Some are integrase inhibitors and cell entry inhibitors.

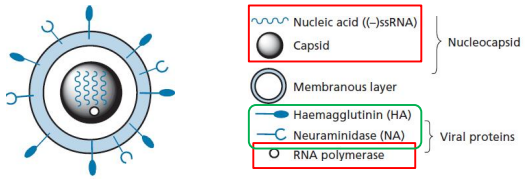
120

Antiviral drugs used against RNA viruses (flu virus)

Structure and life cycle of the influenza virus

- **Influenza (or flu) is an airborne, respiratory disease caused by an RNA virus which**
  - **infects the epithelial cells of the upper respiratory tract.**
- **It is a major cause of mortality,**
  - **especially among the elderly**
  - **or among patients with weak immune systems.**

121



Diagrammatic representation of the flu virus

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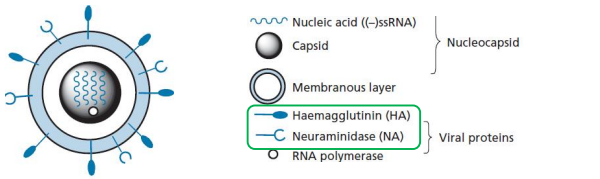
1. **The nucleocapsid of the flu virus contains (-)ssRNA and a viral enzyme called RNA polymerase.**
2. **There is a membranous envelope derived from host cells which contains two viral glycoproteins called neuraminidase (NA) and haemagglutinin (HA).**

The upper respiratory tract consists of a layer of protective mucus . They are rich in glycoproteins and glycolipids which bear a terminal sugar substituent called sialic acid.

122



Diagrammatic representation of the flu virus

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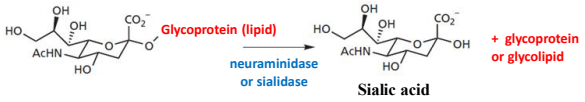
NA (also called sialidase) is an enzyme which cleaves sialic acid from these glycoproteins and glycolipids. thus degrading the mucus layer and allowing the virus to reach the surface of epithelial cells.



Sialic acid

123

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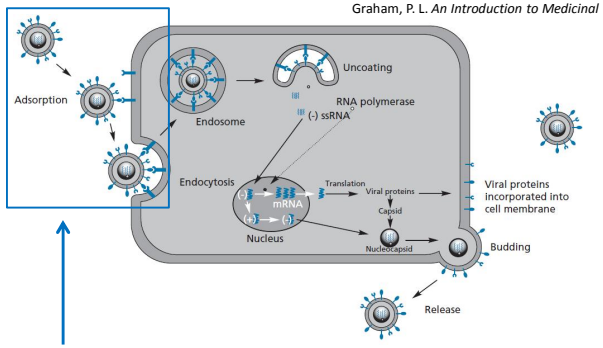

Adsorption takes place whereby the virus binds to cellular glycoconjugates that are present in the host cell membrane, and which have a terminal sialic acid moiety.

The viral protein HA is crucial to this process.

Like NA, it recognizes sialic acid but HA binds to it.

124

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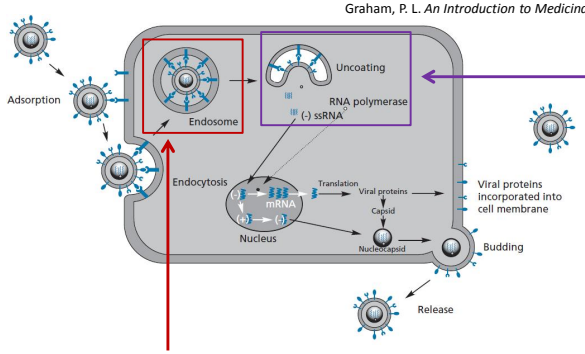

Once the virion has been adsorbed, the cell membrane bulges inwards taking the virion with it to form a vesicle called an endosome — a process called receptor mediated endocytosis.

The pH in the endosome then decreases, causing HA in the virus envelope to undergo a dramatic conformational change .

Then fusion occurs and the RNA nucleocapsid is released into the cytoplasm.

125

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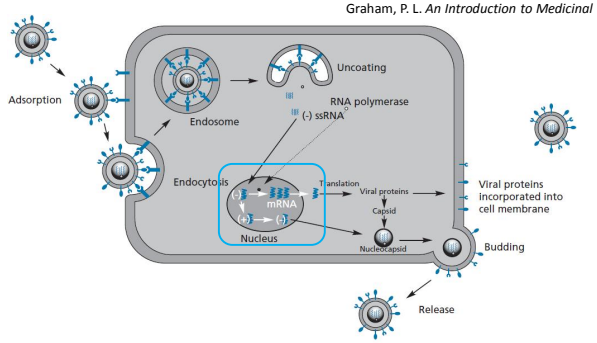

Viral RNA polymerase now catalyses the copying of (−)viral RNA to produce (+)viral RNA, which departs the nucleus and acts as the mRNA required for the translation of viral proteins.

Copies of (−) viral RNA are also produced in the nucleus, then exported into the cytoplasm.

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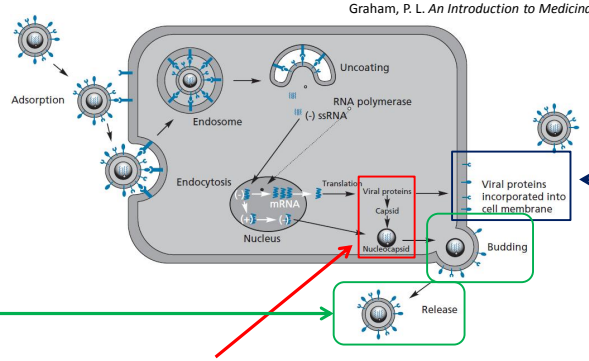

Capsid proteins spontaneously self-assemble in the cytoplasm with incorporation of (−) RNA and newly produced RNA polymerase to form new nucleocapsids.

The freshly synthesized viral proteins HA and NA are incorporated into the membrane of the host cell.

Newly formed nucleocapsids then move to the cell membrane and attach to the inner surface. HA and NA move through the cell membrane to concentrate at these areas and host cell proteins are excluded. Budding then takes place and a new virion is released.

127

- **As HA and NA are on the outer surface of the virion,**
  - **they can act as antigens.**
  - **i.e. molecules which can be potentially recognized by antibodies and the body's defence systems.**
- **It should be possible to prepare vaccines which will allow the body to gain immunity from the flu virus.**
- **There are three groups of flu virus.**
  - **influenza A**
  - **influenza B**
  - **influenza C**

128

Ion channel disrupters

- **Amantadine and rimantadine are related adamantanes with similar mechanisms of action and can inhibit viral infection in two ways.**
  - **At low concentration (<1 µg/ml)**
    - ✓ they inhibit the replication of influenza A viruses by blocking a viral ion channel protein.
  - **At high concentration (>50 µg/ml)**
    - ✓ they buffer the pH of endosomes to prevent the acidic environment needed for HA to fuse the viral membrane with that of the endosome.
    - ✓ Inhibit penetration and uncoating of the virus.

129

Neuraminidase inhibitors

- **Since neuraminidase (NA) has two crucial roles in the infectious process,**
  - **it is a promising target for potential antiviral agents.**
- **Sialic acid was bound to the active site of NA through a network of**
  - **hydrogen bonds and**
  - **ionic interactions.**

130

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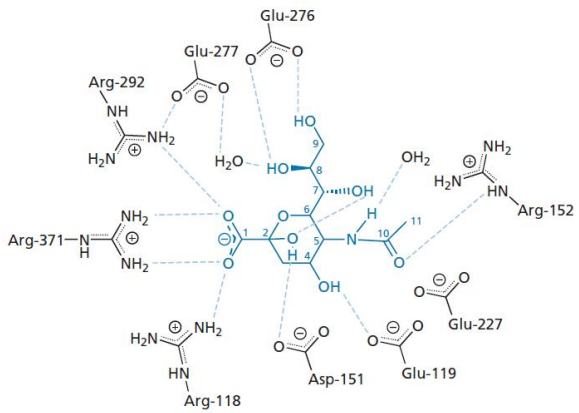
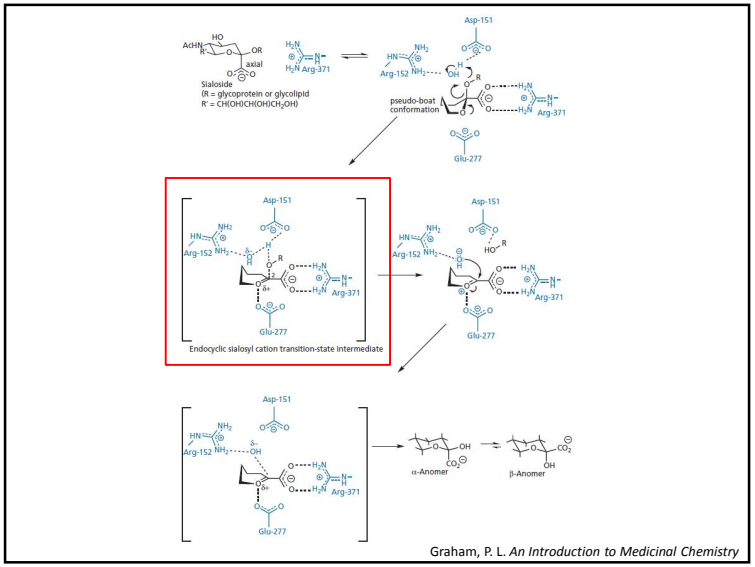


Hydrogen bonding interactions between sialic acid and the active site of neuraminidase

131

- **Transition-state inhibitors,**
  - **2-deoxy-2,3-dehydro-N-acetylneuraminic acid (Neu5Ac2en) was discovered.**
- **Inhibitors introduce an endocyclic double bond to mimic this transition state.**
- **Other transition-state inhibitors**
  - **4-Amino-Neu5Ac2en**
  - **6-carboxamides**

132



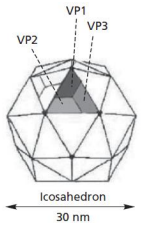
133

Antiviral drugs used against RNA viruses (cold virus)

- The agents used against flu are ineffective against colds
  - as these infections are caused by a different kind of virus called
  - rhinovirus.
- Colds are less serious than flus.
- There are at least 89 serotypes of human rhinoviruses (HRV).

134

- They are among the smallest of the animal RNA viruses
  - containing a positive strand of RNA
  - coated by an icosahedral shell made up of 60 copies of four distinct proteins
  - made up of 60 copies of four distinct proteins,
  - VP1 – VP4.



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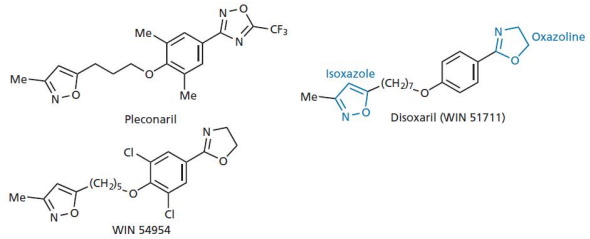
- At the junction between each VP1 and VP3 protein
  - there is a broad canyon 25 Å deep and
  - this is where attachment takes place between the virus and the host cell.
- On the canyon floor there is a pore which opens into a
  - a hydrophobic pocket within the VP1 protein.
- This pocket is either empty or occupied by a small molecule called
  - pocket factor.

136

- When the virus becomes attached to the host cell
  - receptor molecule on the host cell fits into the canyon
  - cause the VP4 protein and the N-terminus of VP1 to move to the exterior of the virus.
- An important process by which the virus is uncoated and releases its RNA into the host cell.
- The pocket factor stabilizes the capsid when it is bound.
- A variety of drugs having antiviral activity are thought to mimic the pocket factor by
  - by displacing it and binding to the same pocket.

137

- The drugs concerned are called capsid-binding agents.
- Examples
  - Pleconaril
  - Disoxaril



Capsid binding agents

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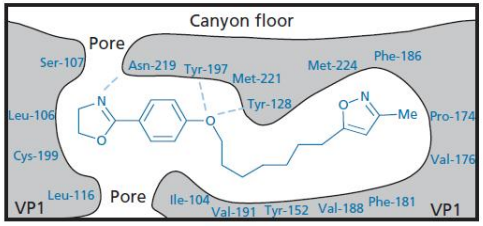

Binding of disoxaril

139